

myeloid-derived cells (CD34<sup>+</sup>CD33<sup>+</sup>CD13<sup>+</sup>CD11b<sup>+</sup>CD15<sup>-</sup>), which are common features of human tumors, and have been linked to poor prognosis in patients with cancer (T L Whiteside, The tumor microenvironment and its role in promoting tumor growth, *Oncogene* (2008) 27, 5904-5912). Under normal conditions, T<sub>reg</sub> cells are involved in the important role of preventing autoimmunity, but in cancer, they expand, migrate to tumors, downregulate autologous effector T-cell proliferation and suppress anti-tumor responses of both CD4<sup>+</sup>CD25<sup>-</sup> and CD8<sup>+</sup>CD25<sup>-</sup> T cells using distinct molecular pathways. The T<sub>reg</sub> cells in the tumor are a heterogeneous population of regulatory CD3<sup>+</sup>CD4<sup>+</sup> T cells, comprising natural T<sub>reg</sub>, antigen-specific Tr1 cells, and other less well defined subsets of suppressor cells. Tr1 cells are induced in the tumor microenvironment, which is rich in IL-10, TGF- $\beta$ , and prostaglandin E<sub>2</sub> (PGE<sub>2</sub>), all of which have been shown to promote Tr1 generation (T L Whiteside, The tumor microenvironment and its role in promoting tumor growth, *Oncogene* (2008) 27, 5904-5912).

**[0009]** Myeloid suppressor cells (MSCs) also suppress T-cell responses in the tumor microenvironment, where they secrete TGF- $\beta$  or induce TGF- $\beta$  secretion. Immunosuppressive CD34<sup>+</sup> cell-derived myeloid cells have been identified in the peripheral blood of cancer patients. In tumor-bearing mice, MSCs accumulate in the spleen and peripheral circulation in very high amounts, exerting potent immunosuppression and favoring tumor growth. MSCs also control the availability of essential amino acids such as L-arginine and produce high levels of reactive oxygen species. The MSCs found in tumors also constitutively express iNOS and arginase 1, an enzyme involved in metabolism of L-arginine, which also synergizes with iNOS to increase superoxide and NO production, which have been found to interfere with lymphocyte responses. GM-CSF, which is also often secreted by tumor cells, recruits MSCs and induces dose-dependent in vivo immune suppression and tumor promotion, while at the same time, GM-CSF has been used as immune adjuvant in antitumor vaccines. GM-CSF was observed to increase a subset of TGF- $\beta$ -producing MSCs in the circulation of patients with metastatic melanoma. The concurrent stimulatory and suppressive roles suggest that GM-CSF and MSCs are involved in maintaining immune homeostasis in normal tissue, but in the tumor microenvironment promote tumor cell escape (T L Whiteside, The tumor microenvironment and its role in promoting tumor growth, *Oncogene* (2008) 27, 5904-5912).

#### Tumor Immunotherapy

**[0010]** Cancer therapy is evolving rapidly as new molecular targets are being discovered. Despite the advent of biologics targeting specific pathways (e.g., Herceptin®, Erbitux®) and small molecules designed against specific targets (tamoxifen, GLEEVEC™), nonspecific modalities such as chemotherapy and radiation remain a standard of care.

**[0011]** Anti-cancer immunotherapy has been a goal for many years with a variety of approaches being tested. One difficulty of developing this immunotherapy is that target antigens are often tissue specific molecules found on both cancer cells and normal cells, and either do not elicit immunity or show non-specificity regarding cell killing (Kaufman and Wolchok eds., *General Principles of Tumor Immunotherapy*, Chpt 5, 67-121 (2007)). Furthermore, tumor cells have features that make immune recognition

difficult, such as loss of expression of antigens that elicit immune response, lack of major histocompatibility (MHC) class II, and downregulation of MHC class I expression. These features can lead to non-recognition of tumor cells by both CD4<sup>+</sup> and CD8<sup>+</sup> T cells (Kaufman and Wolchok eds., *General Principles of Tumor Immunotherapy*, Chpt 5, 67-121 (2007)). Tumors may also evade detection through active mechanisms, such as the production of immunosuppressive cytokines (Kaufman and Wolchok eds., *General Principles of Tumor Immunotherapy*, Chpt 5, 67-121 (2007)).

**[0012]** DCs generated ex vivo by culturing hematopoietic progenitor cells or monocytes with cytokine combinations have been tested as therapeutic vaccines in cancer patients for more than a decade (Ueno H, et al., *Immunol. Rev.* (2010) 234: 199-212). For example, treatment of metastatic prostate cancer with sipuleucel-T (also known as APC 8015), which is a cellular product based on enriched blood APCs that are briefly cultured with a fusion protein of prostatic acid phosphatase (PAP) and granulocyte macrophage colony-stimulating factor (GM-CSF), resulted in an approximately 4-month-prolonged median survival in Phase III trials (Higano C S, et al., *Cancer* (2009) 115: 3670-3679; Kantoff P W, et al., *N. Engl. J. Med.* (2010) 363: 411-422). This study concluded that DC-based vaccines are safe and can induce the expansion of circulating CD4<sup>+</sup> T-cells and CD8<sup>+</sup> T-cells specific for tumor antigens. As a result of this and similar studies, sipuleucel-T has been approved by the US Food and Drug Administration (FDA) for the treatment of metastatic prostate cancer, thereby paving the clinical development and regulatory path for the next generation of cellular immunotherapy products (Palucka K and Banchereau J, *Nature Reviews Cancer* (April 2012) 12: 265-276).

**[0013]** Vaccination strategies involving DCs to induce tumor-specific effector T cells that can reduce the tumor mass specifically and that can induce immunological memory to control tumor relapse have been developed. For example, DCs can be provided with tumor-specific antigens by culturing DCs ex vivo with an adjuvant and a tumor-specific antigen, and then injecting these cells back into the patient. Tumor cells obtained from an excised tumor, needle biopsy, core biopsy, vacuum-assisted biopsy or peritoneal lavage have been used to generate immunogenic compositions comprising tumor-specific-antigen presenting dendritic cells.

#### Cancer Treatment Strategies

**[0014]** Antibody therapies such as Herceptin™ and Erbitux™ are passive immunotherapies, but have yielded considerable improvement in clinical outcome, as measured by, e.g. the recurrence rate, progression free survival and overall survival. More recently, PD-1 and CTLA4 inhibitors have been reported to block discrete checkpoints in an active host immune response allowing an endogenous anti-cancer immune response to be sustained. The term “immune checkpoints” refers to the array of inhibitory pathways that are necessary for maintaining self-tolerance and modulating the duration and extent of immune responses to minimize damage to normal tissue. Immune checkpoint molecules such as PD-1, PD-L1, CTLA-4 are cell surface signaling receptors that play an important role in modulating the T-cell response in the tumor microenvironment. Tumor cells have been shown to utilize these checkpoints to their benefit by up